ACTA PATH MICROBIOL SCAND 52(3): 119-132. 1961

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# PRESENT STATUS OF LABORATORY STUDIES ON TOBACCO CARCINOGENESIS

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Received 5.ix.60

Definitive proof that smoking is a cause of cancer in man rests upon epidemiological studies. Statistical surveys, both of the retrospective and prospective type, have shown without exception that smoking, particularly of cigarettes, has a high association with cancer of the lung. In the opinion of several public health authorities the association is regarded as causative (1, 3, 7, 9, 10, 18, 20, 23).

The basic significance of laboratory studies does not lie in adding to the proof that smoking is a cause of cancer in man, but rather in defining the various steps involved in tobacco carcinogenesis and in permitting studies leading to safer smoking products. It is along these lines that our research program is directed. The present report concerns the current status of laboratory research in this field.

## REDUCTION IN SMOKE CONDENSATE

Statistical studies have shown that the risk of developing lung cancer increases with the amount of tobacco consumed (4, 5, 8). Similarly, biological experiments have demonstrated that the number of tumors obtained in mice is related to the amount of tobacco smoke condensate applied (26). For these reasons studies were undertaken for possible ways of reducing the amount of smoke condensate of cigarettes.

*Filter Cigarettes:* A previous publication showed major differences in smoke condensate in different types of American cigarettes (25). Among the ten leading American cigarettes the amount of smoke condensate ranged from 39.8 mg to 17.7 mg per cigarrette. In general, king size cigarrettes (85 mm) were found to be higher in smoke condensate and nicotine content than regular cigarettes (70 mm) when smoked under standard conditions to the same butt length (23 mm) and cigarettes with filter tips were found to be lower than plain cigarettes. A reduction in smoke condensate can also be achieved through tobacco selection and high porosity or perforated paper.

These studies indicated that the presently used filter materials do not selectively remove carcinogens or promoting substances from to-

bacco smoke condensate. However, in view of the fact that most filter cigarettes tend to reduce the total smoke condensate, provided a smoker does not significantly increase his smoking habits, cigarettes with effective filters represent a safer smoking product than plain cigarettes.

*Butt Length:* Studies determining the smoke condensate and nicotine content of the first and second half of cigarettes showed that the second half of the cigarette contains 41 to 45 per cent more smoke condensate than the first half and the nicotine amount rises similarly (25).

*Puff Frequency:* Studying the puff frequency per cigarette indicated that the amount of smoke condensate markedly increases with the frequency of puffs per cigarette (25). Smoking one of the 85 mm cigarettes, once a minute, gave a reading of 35.1 mg of smoke condensate, twice a minute—53.0 mg, and three times a minute—64.1 mg.

*Inhalation:* Studying the extent of inhalation by measuring the variations of inhaled and not inhaled aerosols of cigarette smoke showed that if the smoker takes the smoke only into his mouth for approximately two seconds, about 10 per cent of the aerosols are retained. On the other hand, a smoker who deeply inhales will retain up to 90 per cent of the smoke aerosols (25).

In summary, clinical laboratory studies indicate that safer smoking would include the use of a type of cigarette, which is as low in smoke condensate and nicotine content as is commensurate with smoking satisfaction, which essentially means an effective filter cigarette. They also suggest that cigarettes should not be puffed too frequently or smoked to the butt and that the smoke should not be deeply inhaled.

## THE IMPORTANCE OF POLYCYCLIC HYDROCARBONS

On the basis of previously published biological and chemical studies, it has been proposed that the identified carcinogenic polycyclic hydrocarbons represent a major group of initiating carcinogens in tobacco smoke condensate (17, 24). The polynuclear aromatic hydrocarbons are mainly formed during the combustion of the tobacco. The tobacco of our standard cigarettes contains only very minute quantities of benzo(a)pyrene (0.02 ppm). A bioassay indicates that these polycyclic hydrocarbons of the condensate by themselves, however, can account for not more than 3 per cent of the total biological activity; and on the basis of the most potent single fraction from the condensate, in which the aromatic hydrocarbons are enriched more than 50 times (Fraction B, Fig. 1), these compounds can account for only 10 per cent of the established biological activity. It has, therefore, been clear that additional carcinogens or co-carcinogens have to be present. Fig. 1 shows the procedures currently in use for chemical separation of cigarette smoke condensate.

where most of the short term activity resides. We are now in the process of separating this fraction into the polycyclic hydrocarbon fraction and other materials.

## PREVENTIVE MEASURES

It has been claimed that one could not establish the carcinogenic activity of a given substance unless one had isolated specific carcinogens. This is an ill-founded view. If a given material produces cancer for a given animal tissue, this proves by definition that this material is carcinogenic, and whether or not one can identify the specific carcinogen does not alter this fact. Our reason for searching for the specific carcinogens, or co-carcinogens, in tobacco smoke condensate has not been to give more credance to the human and animal findings, but rather to permit upon identification of these substances to study their possible reduction. Within our present knowledge we can account for a majority of the established biological activity of tobacco smoke condensate through a combination of initiating carcinogens and promoting substances.

The biological results of different smoke condensates suggest the presence of initiating carcinogens as well as promoting substances. The next step must be to attempt a reduction of both these groups. Proper advance in either direction should reduce the total activity of tobacco smoke condensate.

As stated, the major purpose of animal evidence is to define the mechanism for a better understanding of carcinogenesis and in particular to define the specific substances responsible for the carcinogenic activity. In view of the data at hand, the following preventive measures, short of giving up smoking altogether, which obviously would be the most complete measure, are in order:

(1) Reduction in exposure to smoke condensate through: (a) Modification of smoking habits; (b) Cigarettes with resulting lower content of smoke condensate; (c) Avoidance of deep inhalation, smoking cigarettes to the end, and of drawing on a cigarette too frequently.

(2) Further attempts to reduce the initiating carcinogens through the use of additives. Present attempts in this direction are promising. Such additives may not only decrease polycyclic hydrocarbons but possibly also promoting substances, as found for the phenolic fraction from cigarettes with calcium carbonate and copper(II)nitrate as additives. Attempts have also been made to study precursors of the polycyclic hydrocarbons with the hope that some precursors are particularly susceptible to form these substances (29). We find, however, that most organic materials yield polycyclic hydrocarbons during pyrolysis, especially above 700° C. Also, it does not seem possible to remove selectively the polycyclic hydrocarbons through filtration.

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